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# BLOOD CHANGES AND ANTIBODY PRODUCTION IN HUMAN BEINGS AFTER INJECTION OF PNEUMOCOCCUS LIPOVACCINE *

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Epidemics of acute respiratory diseases occurring during the last few years have caused many attempts to immunize against the organisms isolated in these infections, with the hope that results comparable to the immunization against B. typhosus might be obtained. Among the various vaccines tried a pneumococcus lipovaccine was used extensively in some of the army camps and to a lesser extent among civilians.

In March, 1919, for the purpose of experiment, I inoculated 3 healthy men with the army pneumococcus lipovaccine. One c c of this vaccine contained 0.83 mgm. of the following: types 1, 2 and 3 dried pneumococcal protein. The dose was 1 c c injected subcutaneously. All 3 men had moderately severe local reactions and general constitutional symptoms, consisting of slight rise in temperature, headache, backache, muscular pain, and general malaise. Blood counts were taken on each person at 2 to 3 day intervals for several weeks, then at longer intervals, No. 1 for 3 months, No. 2 for 6 months, and No. 3 for 1 year. At the same time, blood was taken aseptically from the median vein of each person. The serum was collected from the blood, inactivated by heating at 56 C. for 30 minutes, and examined for agglutinin for each pneumococcus type. Selected serums were tested for complement-fixing antibodies and for protective bodies. The pneumococci used for the test were types 1, 2 and 3, which were furnished by the Rockefeller Institute for Medical Research.

## BLOOD

There was no apparent change in the blood picture that could be attributed to the vaccine. In No. 1 (table 1) the leukocytic count dropped from 16,000 to 14,200; in No. 2 (table 2) it rose from 7,650 to 9,450; and in No. 3 (table 3) it remained constant, about 10,000.

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In all three cases, after the injection of the vaccine, there was an increase in the percentage of polymorphonuclear leukocytes and of the eosinophiles, the latter rising from 3% to 10% in No. 1.

## AGGLUTININ

The macroscopic agglutination test was used with different dilutions of inactive serum and a constant amount of an 18-hour culture. The mixtures were incubated for 2 hours at 37 C., then placed in the icebox over night before the readings were noted. Several normal serums and a known positive serum as controls were set up with each test.

Before vaccination, serum from No. 1 and No. 2 contained no demonstrable agglutinins for any of the 3 types of pneumococci. Serum from No. 3 in a 1 : 4 dilution agglutinated type 3 pneumococcus.

TABLE 1

J. A. H.—Findings After Vaccine Injection March 14, 1919

| Date | Blood Count | | | | | | | Agglutinin for Pneumococcus | | | Complement Fixation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | White Blood Cells | Red Blood Cells | Differential | | | | | Type 1 | Type 2 | Type 3 | |
| | | | P., % | L., % | E., % | B., % | L. M., % | | | | |
| 3/14/19 | 16,000 | 4,000,000 | 68 | 22 | 3 | 1 | 5 | 0 | 0 | 0 | 0 |
| 3/17/19 | 14,200 | 4,400,000 | 79 | 16 | 0 | 1 | 4 | 0 | 0 | 2 | |
| 3/19/19 | 12,000 | 4,200,000 | 57 | 31 | 10 | 0 | 2 | 0 | 0 | 4 | |
| 3/21/19 | 12,000 | 4,200,000 | 63 | 33 | 2 | 0 | 2 | 4 | 2 | 8 | 0 |
| 3/24/19 | 7,000 | 4,000,000 | 62 | 34 | 3 | 0 | 1 | 4 | 2 | 8 | |
| 3/25/19 | 6,400 | 4,800,000 | 75 | 21 | 2 | 0 | 2 | 512 | 512 | 32 | + |
| 4/ 1/19 | 8,100 | 3,910,000 | 69 | 25 | 2 | 0 | 6 | 512 | 512 | 64 | |
| 4/ 3/19 | 6,650 | 3,100,000 | 68 | 20 | 4 | 3 | 4 | 512 | 512 | 256 | +++ |
| 4/ 7/19 | 8,250 | 4,160,000 | 69 | 22 | 1 | 0 | 8 | 512 | 512 | 512 | |
| 4/18/19 | 6,250 | 4,990,000 | 65 | 28 | 0 | 0 | 6 | 512 | 512 | 512 | +++ |
| 4/24/19 | 9,100 | 4,900,000 | 56 | 40 | 1 | 0 | 3 | 256 | 512 | 256 | |
| 4/30/19 | 8,700 | 5,100,000 | 66 | 31 | 1 | 0 | 2 | 40 | 40 | 40 | |
| 5/14/19 | 9,850 | 5,120,000 | 63 | 28 | 5 | 0 | 4 | 128 | 128 | 128 | |
| 5/23/19 | 7,250 | 4,000,000 | 65 | 32 | 0 | 1 | 2 | 10,000 | 1,280 | 25,000 | ++++ |
| 6/ 4/19 | 8,650 | 4,420,000 | 67 | 31 | 1 | 0 | 1 | 10,000 | 1,280 | 5,000 | |
| 6/30/19 | 7,750 | 3,770,000 | 70 | 22 | 1 | 0 | 7 | 10,000 | 1,280 | 5,000 | ++++ |

P. = Polymorphonuclear leukocytes
L. = Lymphocytes
E. = Eosinophiles
B. = Basophiles
L. M. = Large mononuclears
+ = Slight inhibition of hemolysis
+++ = Partial inhibition of hemolysis
++++ = Complete inhibition of hemolysis
Figures under agglutinin indicate the highest dilution of serum agglutinating.

Serum 1 (table 1) 7 days after vaccination, in 1 : 4 dilution, agglutinated pneumococcus type 1; in 1 : 2 dilution, pneumococcus type 2 and in 1 : 8 dilution, pneumococus type 3. Normal serum, however, in these dilutions occasionally agglutinated pneumococci, so that it was not until the eleventh day that agglutinins were unmistakably increased above the normal. At this time a 1:512 serum dilution agglutinated types 1 and 2, and 1:32 agglutinated type 3. Types 1 and 2 remained almost constant for a month, and agglutinin for type 3 gradually increased to 1:512. On the forty-fourth day, there was a sudden drop to 1:40 for all 3 types. There was no constitutional disturbance that coincided with this agglutinin decrease, and there was only a slight change in the blood picture. The next observation was made 2 weeks later, when all types were agglutinated by 1:128. The height of agglutinin production, 1:10,000 for type 1, 1:1,280 for type 2, and 1:25,000 for type 3, was reached in another week, 69 days after inoculation. The titer was still high when the final observation, 106 days after vaccination, was taken. At that time agglutinin for type 1 was demonstrable in 1:10,000 serum dilution, for type 2 in 1:1,280 dilution, and for type 3 in 1:1,280 dilution.

TABLE 2

W. J.—FINDINGS AFTER VACCINE INJECTION MARCH 14, 1919

| Date | Blood Count | | | | | | | Agglutinin for Pneumococcus | | | Complement Fixation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | White Blood Cells | Red Blood Cells | Differential | | | | | Type 1 | Type 2 | Type 3 | |
| | | | P., % | L., % | E., % | B., % | L.M., % | | | | |
| 3/14/19 | 7,750 | 4,200,000 | 61 | 38 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| 3/16/19 | 9,450 | 4,950,000 | 70 | 27 | 0 | 0 | 3 | 2 | 1 | 2 | |
| 3/18/19 | 6,900 | 4,200,000 | 76 | 23 | 2 | 0 | 0 | 2 | 2 | 2 | |
| 3/20/19 | 5,300 | 3,990,000 | 55 | 40 | 1 | 0 | 4 | 16 | 2 | 4 | + |
| 3/22/19 | 6,350 | 4,150,000 | 69 | 26 | 2 | 1 | 2 | 16 | 2 | 4 | |
| 3/24/19 | 6,050 | 4,450,000 | 58 | 41 | 1 | 0 | 0 | 16 | 2 | 4 | |
| 3/26/19 | 9,400 | 4,000,000 | 68 | 28 | 0 | 0 | 4 | 640 | 320 | 160 | |
| 3/28/19 | 6,700 | 4,590,000 | 64 | 28 | 3 | 0 | 5 | 1,280 | 320 | 320 | +++ |
| 3/30/19 | 8,300 | 4,380,000 | 70 | 27 | 1 | 1 | 1 | 640 | 320 | 320 | |
| 4/ 2/19 | 6,850 | 4,820,000 | 52 | 43 | 2 | 0 | 3 | 640 | 640 | 320 | ++ |
| 4/ 9/19 | 6,300 | 4,450,000 | 60 | 33 | 4 | 0 | 3 | 20 | 1,280 | 1,280 | ++++ |
| 4/15/19 | 8,850 | 4,420,000 | 39 | 57 | 2 | 0 | 2 | 1,280 | 1,280 | 2,560 | |
| 4/25/19 | 5,700 | 4,640,000 | 50 | 44 | 1 | 1 | 4 | 640 | 1,280 | 1,280 | |
| 5/ 9/19 | 10,600 | 4,810,000 | 68 | 29 | 0 | 0 | 3 | 10 | 1,280 | 1,280 | ++++ |
| 6/ 4/19 | 8,400 | 4,800,000 | 61 | 33 | 1 | 0 | 5 | 640 | 2,560 | 160 | |
| 8/28/19 | 9,200 | 4,900,000 | 65 | 34 | 0 | 1 | 0 | 320 | 1,280 | 320 | ++++ |

P. = Polymorphonuclear leukocytes
L. = Lymphocytes
E. = Eosinophiles
B. = Basophiles
L. M. = Large mononuclears

+ = Slight inhibition of hemolysis
++ = Partial inhibition of hemolysis
+++ = Partial inhibition of hemolysis
++++ = Complete inhibition of hemolysis

Figures under agglutinin indicate the highest dilution of serum agglutinating.

Serum 2 (table 2) agglutinated in low dilutions for 10 days after inoculation. On the twelfth day the agglutinin titer suddenly rose to 1:640, 1:320, 1:160 for pneumococcus types 1, 2 and 3 respectively. The height of agglutinin production occurred 31 days after vaccination, when the readings for all three types were all 1:1,280. When the last observation was made, 5½ months after inoculation, agglutinin was present in 1 : 320 serum dilution for types 1 and 3 and in 1 : 1,280 for type 2. On the twenty-sixth and fifty-sixth days there was a drop in agglutinin for type 1. No clinical cause was demonstrable for this drop, though on the fifty-sixth day the leukocyte count was higher than normal for this individual.

TABLE 3

J. H.—Findings After Vaccine Injection March 14, 1919

| Date | Blood Count | | | | | | | Agglutinin for Pneumococcus | | | Complement Fixation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | White Blood Cells | Red Blood Cells | Differential | | | | | Type 1 | Type 2 | Type 3 | |
| | | | P., % | L., % | E., % | B., % | L. M.. % | | | | |
| 3/14/19 | 10,200 | 4,800,000 | 74 | 19 | 1 | 1 | 5 | 0 | 0 | 4 | 0 |
| 3/16/19 | 10,500 | 6,000,000 | 77 | 16 | 6 | 0 | 1 | 32 | 0 | 2 | |
| 3/18/19 | 10,200 | 5,600,000 | 58 | 30 | 6 | 1 | 5 | 16 | 0 | 8 | |
| 3/21/19 | 8,400 | 4,900,000 | 68 | 27 | 5 | 0 | 0 | 32 | 0 | 64 | 0 |
| 3/23/19 | 7,700 | 4,600,000 | 74 | 20 | 0 | 0 | 6 | 16 | 0 | 64 | |
| 3/25/19 | 8,100 | 4,800,000 | 73 | 21 | 3 | 2 | 1 | 200 | 64 | 400 | |
| 3/27/19 | 10,450 | 4,940,000 | 71 | 24 | 3 | 0 | 2 | 32,768 | 64,000 | 32,768 | ++ |
| 3/29/19 | 10,650 | 4,500,000 | 69 | 22 | 2 | 0 | 7 | 32,768 | 128,000 | 64,000 | |
| 4/ 1/19 | 9,900 | 4,760,000 | 75 | 18 | 3 | 0 | 4 | 32,768 | 512 | 512 | ++ |
| 4/ 4/19 | 7,650 | 4,700,000 | 68 | 28 | 4 | 0 | 0 | 640 | 1,280 | 640 | |
| 4/ 9/19 | 7,600 | 4,000,000 | 68 | 28 | 2 | 0 | 2 | 640 | 2,520 | 640 | |
| 4/15/19 | 7,950 | 4,380,000 | 56 | 40 | 4 | 0 | 0 | 5,040 | 5,040 | 5,040 | |
| 4/23/19 | 8,350 | 4,180,000 | 63 | 34 | 3 | 0 | 0 | 640 | 1,280 | 2,520 | |
| 4/30/19 | 8,000 | 4,500,000 | 65 | 32 | 1 | 0 | 1 | 320 | 640 | 640 | ++++ |
| 5/ 6/19 | 8,200 | 4,780,000 | 67 | 30 | 1 | 0 | 2 | 1,280 | 1,280 | 640 | |
| 5/13/19 | 11,800 | 4,200,000 | 63 | 33 | 2 | 1 | 1 | 320 | 5,040 | 2,500 | |
| 5/23/19 | 9,600 | 4,300,000 | 60 | 39 | 1 | 0 | 0 | 2,500 | 5,400 | 20,000 | ++++ |
| 6/13/19 | 8,560 | 5,000,000 | 62 | 42 | 0 | 1 | 5 | 5,400 | 5,400 | 20,000 | |
| 6/27/19 | 7,800 | 4,080,000 | 60 | 33 | 2 | 1 | 4 | 5,400 | 5,400 | 10,000 | ++++ |
| 7/15/19 | 7,500 | 5,100,000 | 75 | 20 | 0 | 0 | 5 | 10,800 | 5,400 | 10,000 | |
| 10/ 3/19 | 8,200 | 5,100,000 | 60 | 28 | 7 | 1 | 4 | 5,400 | 5,400 | 10,000 | |
| 11/12/19 | 10,400 | 3,400,000 | 67 | 20 | 2 | 0 | 11 | 64 | 64 | 64 | |
| 2/20/20 | 8,500 | 4,900,000 | 65 | 28 | 2 | 1 | 4 | 64 | 64 | 32 | ++ |

P. = Polymorphonuclear leukocytes
L. = Lymphocytes
E. = Eosinophiles
B. = Basophiles
L. M. = Large mononuclears
+ = Slight inhibition of hemolysis
++ = Partial inhibition of hemolysis
++++ = Complete inhibition of hemolysis

Figures under agglutinin indicate the highest dilution of serum agglutinating.

Serum 3 (table 3) contained agglutinin for type 3 in a 1 : 4 dilution before the man received the pneumococcus lipovaccine. Agglutinins for types 1 and 3 were produced at once, and agglutinins for type 2 after 11 days. In 13 days the titer for all types was unusually high, and in 15 days the highest point was reached. On this date, types 1, 2 and 3 were agglutinated by the following serum dilutions: 1 : 32,000, 1 : 28,000 and 1 : 64,000. There were variations in agglutinin content, but the level remained high for 7 months. The serum in the seventh month agglutinated types 1 and 2 in 1 : 5,400 dilution and type 3 in 1 : 10,000 dilution. The next observation made 6 weeks later showed no agglutination for any pneumococcus type above a 1:64 serum dilution. The final test with this serum was made 11½ months after vaccination. At this time 1:64 serum dilution agglutinated pneumococcus types 1 and 2 and 1:32 serum dilution, type 3.

Six persons who had received the pneumococcus lipovaccine a year previously had their blood tested for pneumococcus agglutinins. The results are tabulated in table 4. All serums had agglutinin for pneumococcus type 1, and serums 1, 4 and 6 for types 2 and 3. Serum 3 was taken from a patient convalescing from a typical case of influenza, uncomplicated by pneumonia.

## COMPLEMENT FIXATION TESTS

A number of serums from each man were examined for complement-fixing immune bodies. Two antigens were used throughout the tests. The first antigen was the army pneumococcus lipovaccine emulsified in alcohol and further diluted in normal salt solution. The second antigen was a suspension in normal salt solution of heated washed pneumococci—types 1, 2 and 3 in equal amounts—obtained from 18-hour broth cultures. The technic was that of the original Wassermann test (one-tenth method), and has been previously described.[1] The readings with the two antigens were practically the same.

Serum taken before the 3 men were vaccinated had no demonstrable complement-fixing bodies. The first fixation of complement occurred in serum 1 in 14 days, in serum 2 in 7 days, and in serum 3 in 15 days. On the twenty-fifth day after inoculation, the 3 serums gave 4 plus fixations. Serums 1 and 2 were still 4 plus when observations were discontinued, 3 and 6 months after vaccination. Serum 3 gave a 4 plus fixation until the ninth month, and at the end of 11½ months, a 2 plus fixation.

## PROTECTIVE EXPERIMENTS

Selected serums were used in protective experiments on white mice. The tests were made according to the method described by Cole and his associates.[2]

Great difficulty was experienced in increasing the virulence of the pneumococci used in the test and in maintaining that virulence after it was obtained. Broth cultures from the heart blood, and from the peritoneal fluid, and peritoneal washings were passed through series of mice. Frequently, when the organism had apparently gained a high degree of virulence, this virulence would suddenly drop, and the succeeding mice fail to die. Several times, for example, when the heart blood was plated, to make sure of the purity of the strain before using it in the protective tests, and when colonies were transferred to broth, all within a period of 24 hours, there was such decrease in the virulence of the organism that further mouse passage was necessary before protective tests could be made.

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[1] Howell, K., Jour. Infect. Dis., 1918, 22, p. 230.

[2] Acute Lobar Pneumonia, Monograph of the Rockefeller Institute, 1917, No. 7.

TABLE 4

Individuals Other Than Those Represented in Tables 1, 2 and 3 Who Had Received Pneumococcus Lipovaccine One Year Previous to Testing

| Cases | Agglutinin for Pneumococcus | | |
|---|---|---|---|
| | Type 1 | Type 2 | Type 3 |
| 1 | 250 | 32 | 128 |
| 2 | 500 | 8 | 0 |
| 3 | 500 | 0 | 0 |
| 4 | 500 | 128 | 64 |
| 5 | 1,000 | 0 | 0 |
| 6 | 1,000 | 16 | 16 |

Figures indicate the highest dilution of serum agglutinating.

In the final tests, the virulence of the strains was not high. The lethal dose of pneumococcus type 1 was 0.00005, of pneumococcus type 2, 0.00001, and of pneumococcus type 3, 0.001. Serums in 0.2 c c amount, before the men were vaccinated and for 9 days after vaccination did not protect mice. Serums for the remaining protective experiments were chosen when the agglutinin titer was high. As a rule, the control set of mice died within 24 hours, and the set of mice with serum in 48 to 72 hours.

## DISCUSSION

Among the reports that have been made within the last 2 years on the immunity reactions after prophylactic pneumococcus vaccine are the following: Whitmore, Fennel, and Peterson[3] inoculated 2 men with a pneumococcus lipovaccine containing types 1 and 2. One man had agglutinin for type 1 in a 1:25 serum dilution, which was still existant after 6 weeks. The serum (1:20 dilution) agglutinated type 2 for 2 weeks. A second man's serum agglutinated type 1 in 1:15 dilution and type 2 in 1:40 dilution. No agglutinins were present in this serum after 6 weeks. Fennel[4] found that 10 weeks after injection with the pneumococcus lipovaccine 0.2 c c of serum of an inoculated person protected a mouse against a fatal dose of pneumococci. Cecil and Austin[5] demonstrated agglutinin in 27 of 42 individuals 8 days after vaccination with the pneumococcus lipovaccine and found their serum afforded a definite degree of protection to white mice. Whitmore found that agglutinin was present in the serum of vaccinated men after the eighth day and that the immunity curve rose steadily after that time and continued a considerable period. Blake and Cecil[7] vaccinated a series of monkeys with a pneumococcus lipovaccine, and their serums tested 2 weeks after injection failed to show demonstrable agglutinin and protective bodies. In later experiments, Cecil and Blake[8] by subcutaneously injecting living virulent pneumococcus type 1 produced some agglutinin response when the blood was tested after 2 weeks. Serum from these monkeys taken 20 days after vaccination protected white mice against 1,000 times the minimal lethal dose of pneumococcus type 1. Cecil and Blake in their series of experiments found that sometimes when immune bodies were not demonstrable, monkeys vaccinated with pneumococcus type 1 were still protected against pneumonia of the homologous type and even at times against the other fixed types of pneumonia. They conclude from their experiments that active immunity against pneumonia was largely independent of the presence or absence of agglutinins and protective bodies in the serum of monkeys. Hitchens[9] also decided that immunity did not always coincide with the presence of immune bodies in the serum after pneumococcus vaccination.

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[3] Jour. Am. Med. Assn., 1918, 70, p. 427.

The protective results of pneumococcus vaccine were noted at two large army camps. For example, at Camp Custer, Cecil and Austin[10] found that among the 12,519 vaccinated men there were no cases of pneumonia of the 3 fixed types during the 10 weeks after vaccination, while among the 20,000 unvaccinated men there were 26 cases during the same period. At Camp Wheeler Cecil and Vaughan[11] gave prophylactic pneumococcus lipovaccines to 13,460 men, 80%

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[4] Jour. Am. Med. Assn., 1918, 71, p. 2115.

[5] Jour. Exper. Med., 1918, 28, p. 19.

[6] Harvey Lecture, Jan. 11, 1919.

[7] Jour. Exper. Med., 1920, 31, p. 519.

[8] Jour. Exper. Med., 1920, 31, p. 657 and p. 685.

[9] Med. Rec., 1919, 96, p. 692.

[10] Jour. Exper. Med., 1918, 28, p. 19.

[11] Jour. Exper. Med., 1919, 29, p. 457.

of the force. Excluding the cases developing in the first week, only 8 cases of pneumonia produced by the fixed type occurred.

In the case of the few individuals, doctors and nurses, of the Michael Reese Hospital, who were injected with a pneumococcus lipovaccine, no pneumonia developed and in only one case influenza, uncomplicated by pneumonia, developed during the year in which they were under observation. It would appear that immune bodies after lipopneumococcus vaccine develop slowly, not reaching their height for a considerable time, and persist for a duration of at least several months. It would appear therefore that agglutinin might have been demonstrated in many more of the reported cases, if serum had been tested for agglutinin later than the 8 day or 2 week period that most of the observers chose.

## SUMMARY

Blood: After the pneumococcus lipovaccine there was a slight rise in the polymorphonuclear leukocytes and in the eosinophiles.

Agglutinin: There was a definite rise in agglutinin in the 3 serums examined in 11 to 12 days. The height of agglutinin titer was reached on the 25th, 31st and 15th days, respectively. The agglutinin titer remained high for No. 1 for 3 months (last observation); for No. 2 for 6 months (last observation); for No. 3 for 9 months. Demonstrable agglutinin was present in 7 persons for at least a year.

Complement-Fixing Immune Bodies: Complement-fixing antibodies were present in the serum of the 3 men examined on the 14th, 7th, and 15th days, respectively. The height was reached in 25 days, and all 3 serums showed some degree of fixation in the last test made on each serum.

Protective Experiments: Protective bodies for pneumococcus types 1, 2 and 3 were demonstrable in some degree in the 3 serums at the time the agglutinin titer was high.

There was no apparent correlation between the clinical history, the blood count, and the antibody content.

## CONCLUSION

Agglutinins, complement-fixing bodies, and protective bodies for pneumococci types 1, 2 and 3 are demonstrable in the serum of individuals vaccinated with pneumococcus lipovaccine. These antibodies appear in the serum rather late and are present to some degree for at least one year.